

Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx Product Insert

REF Dx55600



IVD

 PIDx55600-3

Intended Use

Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx provides a fast, reliable and simple procedure for isolating cell-free circulating DNA (cfc-DNA) from 1 to 4 mL of plasma/serum for subsequent *in vitro* diagnostic use. Purification is based on spin column chromatography that uses Norgen's proprietary resin as the separation matrix. This kit is designed to isolate all sizes of cfc-DNA from either fresh or frozen plasma/serum samples. Moreover, this kit allows the user to elute the purified cfc-DNA into a flexible elution volume ranging from 25 µL to 50 µL.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques.

Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

| Component | Product # Dx55600 (20 preps) |
|------------------------|------------------------------|
| Binding Buffer B | 2 x 85 mL 1 x 12 mL |
| Proteinase K | 6.5 mL |
| Solution WN | 18 mL |
| Wash Solution A | 18 mL |
| Elution Buffer B | 30 mL |
| Micro Spin Columns | 20 |
| Midi Spin Columns | 20 |
| Collection Tubes | 20 |
| Elution tubes (1.7 mL) | 20 |
| Product Insert | 1 |

Label Legend

| | | | | | | | | |
|---|---|------------|------------------|---|---|------------------------------------|---|---|
|  |  | LOT | REF |  |  | IVD |  |  |
| Do not reuse | Use by | Batch Code | Catalogue Number | Contains sufficient for <n> tests | Manu-facturer | In Vitro Diagnostic Medical Device | Consult instructions for use | Temper-ature limitation |

Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- Fast and easy processing using rapid spin-column format
- High yields of cell-free circulating DNA
- No phenol or chloroform extractions

Specifications

| Kit Specifications | |
|-----------------------------------|--|
| Anti-coagulant (for Plasma) | EDTA, Citrate or Heparin |
| Plasma Preservative | Norgen's cf-DNA Preservative Tubes Dx (Cat. 63950, 63960) Cell-Free DNA BCT [®] (Streck) |
| Sample Volume Range | 1 to 4 mL |
| Minimum Elution Volume | 25 µL |
| Maximum Elution Volume | 50 µL |
| Time to Complete 10 Purifications | 15 - 20 minutes |
| Size of DNA Purified | ≥ 50 bp |
| Average Yields † | Variable depending on specimen |

† Please check page 5-6 for Average Plasma/Serum Yields and Common DNA Quantification Methods

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2.5 years after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Safety Information and Precautions

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as PDF files online at www.norgenbiotek.com.

Binding Buffer B and **Solution WN** contain guanidine hydrochloride (GnHCl), and should be handled with care. GnHCl forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Plasma or serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx:

- Benchtop microcentrifuge
- Swinging bucket centrifuge
- Micropipettors
- 15 mL tubes
- 96 – 100% ethanol
- 60°C water bath

Procedure

Important Notes

- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The spin columns provided with Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Kits are optimized to be used with benchtop centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Micro Spin Columns.
- Most standard swinging bucket centrifuges will accommodate Norgen's Midi Spin Columns. Do not use a fixed-angle rotor
- Norgen's Midi are centrifuged in 15 mL centrifuge tubes.
- Centrifuging Norgen's spin columns at a speed higher than recommended in the procedure may affect DNA yield.
- Centrifuging Norgen's spin columns at a speed lower than recommended in the procedure will not affect DNA yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the spin column
- When placing Norgen's Midi Spin Columns into the swinging bucket centrifuge make sure that lids of the tubes are not tightly closed. Tightly closed lids may cause back pressure which may cause column clogging or disintegration.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Solution WN** by adding 24 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated Solution WN. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.
- Preheat a waterbath or heating block to 60°C.
- Always **vortex** the **Proteinase K** before use.
- ***This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on Heparin, EDTA or citrate.***
- ***If any of the solutions do not go through the Spin Columns within the specified centrifugation time, spin for an additional 1-2 minutes until the solution completely passes through the column. Do NOT exceed the centrifugation speed as this may affect DNA yield.***
- ***Frozen plasma (from Blood collected on Heparin, EDTA or Citrate Tubes) or serum samples should be centrifuged for 2 minutes at 400 x g (~2,000 RPM) before***

processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.

- **VERY IMPORTANT!** *Frozen plasma recovered from Norgen's cf-DNA Preservative Tubes (Cat. 63950, 63960) may contain some precipitates upon thawing. DO NOT discard any precipitates before cfc-DNA purification. Briefly vortex the plasma and proceed immediately for cfc-DNA purification. Discarding any precipitates may significantly lower cfc-DNA yield.*

Note: *The procedure outlined below is for processing 1 mL to 4 mL inputs of Plasma/Serum. If the sample volume is lower than 4 mL Plasma/Serum, simply bring the volume of your sample up to 4 mL using 1X PBS and proceed as outlined below.*

1. Place 4 mL of plasma/serum sample in a 15 mL tube (provided by the user). Add 240 μ L **Proteinase K** and mix well by vortexing for 10 seconds, then incubate at **room temperature for 10 minutes** with mixing by inversion at an interval of 5 minutes.
 2. After incubation, add 7 mL of **Binding Buffer B**, and mix well by vortexing for 10 seconds.
 3. Transfer up to 5.5 mL of the mixture from **Step 2** into a Midi Spin Column assembled with one of the provided collection tubes. Centrifuge for **5 minutes at 2,000 x g (~4,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube. **(Note: Make sure that lid of the tubes is not tightly closed)**
 4. Repeat **Step 3** to transfer the remaining mixture from **Step 2** into the Midi Spin column.
 5. Apply 1 mL of **Solution WN** to the column and centrifuge for **5 minutes at 2,000 x g (~4,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 6. Apply 1 mL of **Wash Solution A** to the column and centrifuge for **5 minutes at 2,000 x g (~4,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 7. Spin the column, empty, for **10 minutes at 2,000 x g (~4,000 RPM)**. Discard the collection tube.
 8. Transfer the Midi Spin column to a fresh 15 mL tube (not provided). Apply 0.25 mL of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **5 minutes at 500 x g (~1,600 RPM)**.
 9. Apply an additional 1 mL of **Elution Buffer B** to the column and let stand at room temperature for 3 minutes. **Centrifuge for 5 minutes at 2,000 x g (~4,000 RPM)**.
 10. To the elution from **Step 9**, add 1.25 mL of **Binding Buffer B** to the mixture and mix well by vortexing for 10 seconds.
 11. Transfer 700 μ L of the mixture from **Step 10** into a Micro Spin column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,300 x g (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 12. Repeat **Step 11** three more times to transfer the remaining mixture into the Micro Spin column.
 13. Apply 500 μ L of **Solution WN** to the column and centrifuge for **1 minute at 3,300 x g (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 14. Apply 500 μ L of **Wash Solution A** to the column and centrifuge for **1 minute at 3,300 x g (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 15. Repeat **Step 14** one more time.
 16. Spin the column, empty, for **2 minutes at 13,000 x g (~14,000 RPM)**. Discard the collection tube.
 17. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 25 - 50 μ L of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x g (~2,000 RPM)**, followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
- **Plasma/Serum DNA is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the DNA, please refer to Appendix A**

Appendix A

Cell-Free Circulating DNA Yield

Plasma/Serum Cell-free circulating DNA (cfc-DNA) is normally found in very low amounts (1 - 100 pg/ μ L), therefore measuring cfc-DNA concentration using common DNA quantification methods is very difficult and challenging. Typical yields of cfc-DNA vary significantly from sample to sample. Variability is also observed between samples collected from the same donor at different times during the day and therefore there is no absolute yield for cfc-DNA purified from bodily fluids including plasma or serum. Cell-free circulating DNA yield varies depending on a number of factors including age, sex, diet, exercise and most importantly the health status of the donor.

Below is a list of the most common DNA quantification methods, as well as the limit of detection for each of these methods. **Unfortunately, none of these methods can be used reliably for measuring the concentration of DNA purified from plasma or serum unless large plasma/serum volumes have been processed.** This would only be applicable if plasma/serum contains the maximum amount of DNA that can fit within the specification range of these quantification tools. It should be noted that the specifications outlined below are based on measuring a pure dsDNA, which will not be the case for the DNA purified from plasma or serum. Plasma/Serum DNA is short fragmented DNA which is usually present in less than 1000 bp. Purified plasma/serum DNA usually contains traces of proteins which will interfere with most quantification methods, leading to the overestimation of the purified DNA concentration. Therefore purified DNA contaminated with more proteins will be presented at a higher concentration as compared to DNA purified with less protein contaminants, which in this case will depend on the method used for plasma/serum DNA purification. ***The only reliable method that can assess the quality and the relative quantity of the purified plasma/serum DNA is qPCR amplification of a standard DNA using a small DNA amplicon such as the 5S rRNA housekeeping gene.***

Common DNA Quantification Methods

1) 2100 Bioanalyzer DNA Quantification Kits

| | DNA 1000 Kit | DNA 7500 Kit | DNA 12000 Kit | High Sensitivity DNA Kit |
|-----------------------|--------------------|--------------------|--------------------|--------------------------|
| Size Range | 25–1000 bp | 100–7500 bp | 100–12000 bp | 50-7000 bp |
| Quantitation accuracy | 20% CV* | 20% CV* | 25% CV* | 20% CV |
| Quantitative range | 0.5-50 ng/ μ L | 0.5-50 ng/ μ L | 0.5-50 ng/ μ L | 5-500 pg/ μ L |

2) NanoDrop 2000

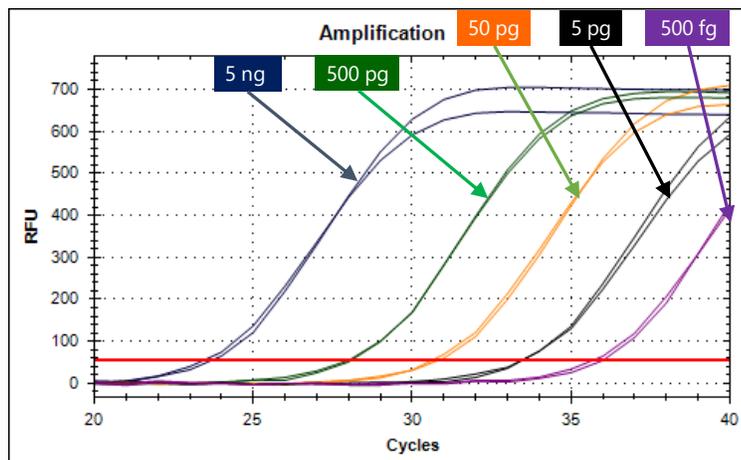
- Detection Limit: 2 ng/ μ L (dsDNA)

3) Quant-iT™ Pico Green® dsDNA Assay Kit

- Detection Limit: 25 pg/mL

4) qPCR DNA Standard Curve

(generated using Norgen's Low Abundance DNA Quantification Kit Cat# 57200)



Frequently Asked Questions

- 1. What if a variable speed centrifuge is not available and the speed differs from the recommended?**
 - A fixed speed centrifuge can be used, however reduced yields may be observed.
- 2. At what temperature should I centrifuge my samples?**
 - All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.
- 3. What if I added more or less of the specified reagents' volume?**
 - Adding more or less than the specified volumes may reduce both the quality and the quantity of the purified DNA. Eluting your DNA in high volumes will increase the yield but will lower the concentration. Eluting in small volumes will increase the concentration but will lower the overall yield.
- 4. What if I forgot to do a dry spin before my final elution step?**
 - Your purified DNA will be contaminated with the Wash Solution A. This may reduce the quality of your purified DNA and will interfere with your downstream applications.
- 5. Can I perform a second elution?**
 - Yes, but it is recommended that the 2nd elution be in a smaller volume (50% of 1st Elution). It is also recommended to perform the 2nd elution into a separate elution tube to avoid diluting the 1st elution.
- 6. What if my incubation temperature varied from the specified 60°C?**
 - The incubation temperature can be in the range of 55°C - 60°C. If the temperature is outside of that range the activity of the Proteinase K will be reduced. This will result in a reduction in your DNA yields.
- 7. What if my incubation time varied from what is specified in the product manual?**
 - Varying the incubation time will result in a reduction in your DNA yields.
- 8. Why do my samples show very low DNA yield?**
 - Plasma/Serum samples contain very little cfc-DNA. This varies from individual to individual. In order to increase the yield, the amount of Plasma/Serum input could be increased.
- 9. Why does my purified cfc-DNA not perform well in downstream applications?**
 - If a different Elution Buffer was used other than the one provided in the kit, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your Elution Buffer with the intended use.
- 10. Do I need to do an RNase treatment for my DNA Elution?**
 - Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Kits do not co-purify circulating RNA along with circulating DNA, therefore an RNase step is not required.
- 11. Why are the A260:280 ratio and the A260:230 ratio of the purified DNA low?**
 - Most of the Plasma/Serum Cell-Free Circulating DNA is present in short fragments. The low A260:280 ratio and the low A260:230 ratio will not affect any downstream applications.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx or NORGEN products in general, please do not hesitate to contact us.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Authorized Representative



EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

 Norgen Biotek Corp.
3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362